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Changes in testicular DNA in mice against immunostimulation and hepatitis

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ABSTRACT

Male swiss albino mice were treated with immunostimulant (@ 150 mg/mouse) (group I), and immunostimulant (@ 150 mg/mouse) + antigen (@ 0.007 ml to 1 ml) (groups A to F). One group (N) of mice was kept as controls (untreated) for comparison. Estimation of testicular DNA was done on day 1,2,3,4 and 5 (on day 11 to 15 of experimental design) of experimental period after immunostimulation and/or antigenation. Mice treated with immunostimulant alone showed significant increase of testicular DNA when compared with controls. However, experimental mice (treated with immunostimulant + antigen, groups A to F) recorded marked alterations (increase or decrease) in the level of DNA when compared with controls and immunostimulated mice. Treatment of mice (experimental) with immunostimulant and antigen resulted in various temporal changes in the DNA content in testes.

Keywords: Immunex DS,Hbs Ag, DNA,Mice.

INTRODUCTION

Hepatitis B is the major cause of morbidity and mortality world-wide. Several attempts have been made to develop an effective and efficient animal model for the complete study of ailment (Sitia et al., 2007; Chayama et al., 2011). Lack of a specific animal model led great gulf to understand the immunopathogenicity of hepatitis B virus infection and its genesis into liver fibrosis and liver carcinoma (Jin et al., 2011). Though vaccines against hepatitis B are available for more than a decade, the infection is still reflecting the difficulties in vaccination (Alter *et al*., 1990; Shapiro and Margolis, 1990). Petrunov *et al.,* (2007) evaluated the role of immunostimulants in immunotherapy and immunoprophylaxis; immunostimulants increase resistance to bacterial and viral infections by stimulating non-specific immunity mechanism. Jung et al., (2015) reported the protection of male reproductive system of young patients from the adverse effects induced by busulfan (cancer agent) by the administration of Korean red ginsing extract. Naghdi et al., (2016) showed that leaf extract of *Ficus carica* improved sperm count and none-progressive motility of spermatozoa of testes of mice intoxicated with formaldehyde. Chemicals and oxidative stress may cause alterations in the protein and DNA levels in testes of mice and rats (Mazur et al.,1994; Kaur and Kaur, 2000; Kaur and Bansol, 2004; Chinoy et al., 2005; Sumedha and Miltonprabu, 2014).

Vawdaw and Mandlwana (2009) reported that protein deficiency diet causes significant reduction in rat testicular DNA, RNA and protein content. Nathanael and Vardhani (2014) and Divya Teja and Viveka Vardhani (2014) stated that the treatment of Immunex DS enhanced the level of liver protein and DNA, and abdominal muscle carbohydrate and glycogen content in mice treated with immunostimulant. Paternal exposure of mice to the environmental contaminent, benzo(a)pyren may reach the testes and cause DNA damage and hypomethylation (Roger et al.,2015). Various studies in human and experimental animals have shown that stress (Priya and Reddy,. 2012; Priya et al., 2014; Arun et al., 2016) and exposure to cyclophosphamide (Quilta., 2016), deltamethrin (Al-Amoudi.,2016), acrylamide (Ahmed and El-Menoufy., 2016) and levofloxacin (antibiotic) may cause adverse effects on the male reproductive system. Male mice treated with immunostimulant, and immunostimulant + HBV antigen recorded enhanced protein level in testes of immunostimulated, and increased/decreased protein content respectively (Jasmin Gold and Viveka Vardhani, 2016b). Recent available literature indicated that exposure to chemicals and oxidative stress effect the testicular morphology and function but the information on the biochemical changes of testis is scarce.

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Therefore, present investigations were aimed to study the effect of hepatitis on testicular DNA during immunostimulation in male mice.

MATERIALS AND METHODS

Male swiss albino mice (*Mus musculus albinus)* (6-8 weeks; 23-32g) fed with standard balanced diet and water *ad libitum* and taken care according to the guidelines of CPCSEA. Eight groups (10 in each group) were employed; six groups of each mice (A,B,C,D,E and F) were orally intubated @ of 150 mg Immunex DS (IDS) on day 0 (single dose) and waited for 72 hours, and vaccinated intramuscularly with various single doses of Gen Vac B Hbs Ag vaccine (A, 0.07 ml/mouse; B, 0.01ml/mouse; C, 0.2ml/mouse; D, 0.4ml/mouse; E, 0.8ml/mouse; F, 1.0ml/mouse). A single group (I) of mice was intubated orally with a single dose of 150mg of IDS/mouse and another group (N) served as controls (without immunostimulant + antigen) for comparison. Two mice from experimental (groups A to F), IDS treated (group I) and controls (group N) were necropsied on day 1, 2, 3, 4 and 5 of experimental period (from day 11 to 15). Testes samples were collected and analyzed for DNA according to Diphenylamine method (Burton, 1956). Results were subjected to Student's T test to understand the statistical significance.

RESULTS AND DISCUSSION

All the experimental mice (except in group B) showed higher level of DNA than the controls and lower level of DNA than the IDS treated ones (group I) (Table-1). Mice of group B showed lower level of DNA compared with control and IDS treated mice; minimum content of DNA (100.5 mg/ml) was found on day 1.

Group A: Higher and lower level of DNA was found when compared to controls (group N) and immunostimulated (group I) mice during the entire experimental period. The content of DNA increased gradually from day 1 (260.0 mg/ml) to 5 (274.0 mg/ml).

Group B: Lower level of DNA was found from day 1 to 5 when compared to controls and immunostimulated mice. Highest amount of DNA was found on day 5 (139.8 mg/ml)

Group C: Increased and decreased level of DNA was found throughout the experimental period compared to controls (group N) and immunostimulated mice (group I).

Group D: Higher and lower value of DNA was noticed throughout the experimental period when compared to control (group N) and immunostimulated (group I) mice. The DNA levels increased gradually from day 1 (239.9 mg/ml) to 5 (246.0 mg/ml).

Group E: Mice of group E showed higher and lower level of DNA when compared with controls (group N) and immunostimulated mice (group I).

Group F: Higher and lower level of DNA was found in mice of group F from day 1 to 5 of infection when compared to controls and IDS treated animals. The DNA level increased gradually from day 1 (360.0 mg/ml) to 5 (372.0 mg/ml).

Mice of group B showed lower level of DNA compared with controls and IDS treated mice; minimum content of DNA (100.5 mg/ml) was found on day 1. In comparison with controls, there was a significant increase of DNA in immunostimulated mice. DNA level showed a significant increase in groups D, E and F when compared with controls (group N) and a significant decrease when compared with IDS treated animals (group I). A significant difference was noted in the level of DNA when compared among themselves (Table-2).

Experimental animals of groups A, B, C, D, E and F (oral administration of immunostimulant as a single dose +antigen) showed significant increase of testes DNA when compared with controls and significant decrease when compared with immunostimulated mice. These results suggest that the treatment of IDS significantly altered the content of DNA in testes. Though the experimental groups (A, B, C, D, E, F) of animals were injected with antigen, the orally administered IDS might have enhanced the level of testes protein and DNA (this

Table-1. DNA (mg/ml) content in the testis of control (group N - untreated with IDS and antigen), IDS treated (group I, @ 150 mg/mouse) and experimental (group A, treated with IDS @ 150 mg/mouse and with Gene Vac B antigen @ 0.07 ml/mouse; group B, treated with IDS @ 150 mg/mouse and Gene Vac B antigen @ 0.1 ml/mouse; group C, treated with IDS @ 150 mg/mouse and Gen Vac B antigen @ 0.2 ml/mouse; group D, treated with IDS @ 150 mg/mouse and Gene Vac B antigen @ 0.4 ml/mouse; group E, treated with IDS @ 150 mg/mouse Gene Vac B antigen @ 0.8 ml/mouse; group F, treated with IDS @ 150 mg/mouse Gene Vac B antigen @ 1 ml/mouse) male swiss albino mice at different days of experimental period. Values are expressed in the mean derived from five observations.

Day of necropsy	Group N	Group I	Group A	Group B	Group C	Group D	Group E	Group F
1	177.3	588.9	260.0	100.5	176.6	239.9	320.0	360.0
2	177.0	588.0	264.0	113.3	241.1	240.0	324.0	364.0
3	177.2	588.2	268.0	116.6	244.0	242.0	328.0	368.0
4	177.3	588.4	270.0	128.2	246.0	244.0	330.0	370.0
5	177.2	588.7	274.0	139.8	248.0	246.0	332.0	372.0

Table-2. 't' values obtained in different experimental groups (A,B,C,D,E and F) of mice

	Experimental Groups						Control Groups	
	A	B	C	D	E	F	N	I
Mean	267.0	119.6	231.1	242.3	326.8	366.8	177.2	588.4
	A N	B N	C N	D N	E N	F N	N I	
							t= 6496.9*	
t value	t= 51.0*	t=9.6*	t= 4.4*	t= 10.7*	t= 5.7*	t= 4.0*		
	A I	B I	C I	D I	E I	F I		
	t= 149.1*	t= 78.6*	t= 29.2*	t= 24.8*	t= 5.2*	t= 10.7*		
	A B	A C	A D	A E	A F			
	t= 23.2*	t= 2.9*	t= 10.5*	t= 20.8*	t= 34.9*			
	B C	B D	B E	B F				
	t= 8.1*	t= 20.0*	t= 33.0*	t= 39.3*				
	C D	C E	C F	D E	D F	E F		
	t= 0.03@	t= 7.7*	t= 0.55@	t= 39.1*	t= 57.8*	t= 14.6*		

P value at 5% level of significance is 2.306; *Statistically significant values; @Statistically non-significant values

enhancement is in comparison with controls). These results compare well with that of Petrunov *et al*., (2007) and Park *et al*., (2008) who also confirmed that immunostimulants are able to reinforce body's natural immunity to cope with various bacterial and viral infections in humans and mice. A significant increase in the DNA content of testes during IDS and Gene Vac B antigen treatment may be associated with the abnormal function of testes as reported by Kaur and Kaur (2000) during selenium treatment in rats, Chinoy et al., (2005) on floride treatment in mice, and Al-Amodi (2016) by deltamethrin and Ahmed and El-Menoufy (2016) by acrylamide toxicity in rats.

All the experimental groups (C, D, E, F) of mice showed increased level of DNA when compared with controls and decreased level of DNA in testes when compared with IDS treated animals. Mice of group A showed increased level of testes DNA when compared with controls and increased level of DNA when compared with IDS treated animals. Testes of mice of group B showed decreased level of DNA when compared with controls and IDS treated animals. Abnormalities in testes DNA are not indicative of the diseased condition but the inoculated various doses of antigen altered the capacity of the reproductive tissue responsible for the balance between protein and DNA synthesis. The increased/decreased level of DNA in testes of mice indicates the disturbed metabolic activity to meet the stress induced by exposure to various doses of antigen. Kaur and Dhanju (2004) and Dhar *et al*., (2014) also suggested that rats exposed to pesticide and fungicide exhibit enhanced metabolic activity in the plasma and testes respectively. These results compare well with that of Sinha *et al.,* (1995) who also found elevation in the activities of specific testicular marker enzymes in rats exposed to endosulfan. Khan and Sinha (1996), Esin (2008), Hatipoglu (2009), Jasmin Gold and Viveka Vardhani (2014, 2016a) and Umar *et al* (2012) also explain that toxicity can cause morphological and functional changes in the male reproductive system of various animals. The higher and lower level of testicular DNA in experimental mice of all the groups (when compared with controls) correlate with the findings of Jasmin Gold and Viveka Vardhani (2016b) who also found marked alteration in the

testicular protein in mice exposed to a single dosage of immunostimulant and varied dosage of antigen.

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Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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